Peptides by Activation of Amino Acids with CO on (Ni,Fe)S Surfaces: Implications for the Origin of Life

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In experiments modeling volcanic or hydrothermal settings amino acids were converted into their peptides by use of coprecipitated (Ni,Fe)S and CO in conjunction with H_2S (or CH_3SH) as a catalyst and condensation agent at 100°C and pH 7 to 10 under anaerobic, aqueous conditions. These results demonstrate that amino acids can be activated under geochemically relevant conditions. They support a thermophilic origin of life and an early appearance of peptides in the evolution of a primordial metabolism.

The activation of amino acids and the formation of peptides under primordial conditions is one of the great riddles of the origin of life. We have now found that under the hot, anaerobic, aqueous conditions of a setting with magmatic exhalations, amino acids are converted into peptides. Under these conditions we previously demonstrated the conversion of carbon monoxide into activated acetic acid in an aqueous slurry of coprecipitated (Ni,Fe)S at 100°C (*1*).

Peptides were formed from phenylalanine (F), tyrosine (Y), and glycine (G). In each run 500 μmol of the amino acid were reacted in a slurry of 1 mmol of FeS and 1 mmol of NiS in 10 ml of water with 4 mmol of CO gas (1 bar) in the presence of 500 μmol of hydrogen sulfide (H_2S) or methanethiol (CH_3SH) at 100°C and pH 7 to 10. In some of the runs 500 μmol of Na_2HPO_4 were added. After 1, 2, or 4 days, we determined the yield of the peptides and the pH in the water phase (*2*) (Table 1). No peptides were detectable, if under otherwise identical conditions CO was replaced by Ar, or if neither H_2S nor CH_3SH was added, or if both NiS and FeS were absent. In runs 13 and 14 and 19 to 22, about 3 nmol of tripeptides (Y-Y-Y) were detected after 1 and 4 days.

In separate experiments it was determined that under these same conditions dipeptides hydrolyzed rapidly. For example, 100 μmol of the dipeptide G-G generated 124 or 48 μmol of G in 1 day at pH 8.4 under otherwise the same conditions as in runs 25 or 27, respectively. This means that the amounts of dipeptides given in Table 1 constitute a balance between reactions of condensation and reactions of hydrolysis. Such a counter-operation of synthetic (anabolic) and hydrolytic (catabolic) reactions with corresponding steady-state concentrations of the products is typical for all extant metabolisms.

The experiments with L-phenylalanine and L-tyrosine produced both epimeric dipeptides as a result of racemization. In the case of L-tyrosine, racemization was extensive after 4 days. These results mean that in an origin of life on (Fe,Ni)S at elevated temperatures, amino acids would be racemic. In a chemoautotrophic origin of life (*3*) with a catalytic feedback of amino acids or short oligopeptides as ligands for catalytic metal centers homochirality of the amino acids or of their peptides is not essential. Homochirality becomes increasingly important with increasing chain lengths of the peptides. It is of interest that the oligopeptides of cell walls have both D– and L–amino acids (*4*).

The results of Table 1 show that the formation of peptides is strongly dependent on the pH of the reaction medium. In the absence of phosphate, significant peptide yields were obtained at pH of about 8 to 9.5. In the

Table 1. Formation of dipeptides from L-phenylalanine (LF), L-tyrosine (LY), D,L-tyrosine (DY,LY) and glycine (G) in the presence of CO, H_2S (or CH_3SH), and 1 mmol of NiS + 1 mmol of FeS. The amount of G-G in run 25 is an average of four reactions with a standard deviation of ±1.5.

Run	H_2S (0.5 mmol)	CH_3SH (0.5 mmol)	Na_2HPO_4 (0.5 mmol)	Days	pH	Dipeptides (μmol)	
L-Phenylalanine (LF)						LF-LF+DF-DF	LF-DF+DF-LF
1	+	–	–	1	7.5	<1	<1
2	+	–	–	1	8.5	7.5	1
3	+	–	–	1	8.8	12	4
4	+	–	–	1	9.6	1	<1
5	–	+	–	1	7.6	<1	<1
6	–	+	–	1	8.5	7	2
7	–	+	–	1	9.2	16	3
8	–	+	–	1	9.9	<1	<1
9	–	+	+	1	7.8	7.6	2
10	–	+	+	1	8.5	12.3	3
11	–	+	+	1	9.1	16.5	3
12	–	+	+	1	9.4	7.1	5
L-Tyrosine (LY)						LY-LY+DY-DY	LY-DY+DY-LY
13	+	–	–	1(4)	9.0(8.0)	15(15)	4.2(14)
14	+	–	–	1(4)	9.1(8.1)	16(15)	5.7(15)
15	+	–	–	1(4)	9.6(8.6)	12(13)	3.4(14)
16	+	–	–	1(4)	9.6(8.7)	9(12)	2.2(13)
17	+	–	–	1(4)	9.8(9.1)	1.8(8.2)	0.5(7.7)
18	+	–	–	1(4)	9.9(9.2)	1.3(6.9)	0.3(6.9)
D,L-Tyrosine (DY, LY)						LY-LY+DY-DY	LY-DY+DY-LY
19	+	–	–	1(4)	9.0(7.9)	10(12)	10(15)
20	+	–	–	1(4)	9.1(7.9)	9(11)	9(14)
21	+	–	–	1(4)	9.4(8.3)	9(12)	11(17)
22	+	–	–	1(4)	9.5(8.4)	11(15)	11(17)
23	+	–	–	1(4)	9.8(9.1)	1.4(7.6)	1.6(10)
24	+	–	–	1(4)	9.8(9.1)	1.2(7.2)	1.5(9)
Glycine (G)						G-G	
25	+	–	–	1	9.2	15	
26	–	+	–	1	7.0	<1	
27	–	+	–	1	8.5	17	
28	–	+	–	1	9.0	21	
29	–	+	–	1	10.1	5	
30	–	+	–	2	8.6	27	
31	–	+	–	2	10.1	6	
32	–	+	+	2	7.0	10	
33	–	+	+	2	8.6	29	
34	–	+	+	2	9.1	31	

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presence of phosphate, the productive pH range was broader. In our reaction system the pH of the aqueous reaction medium decreased with time. Therefore, we followed for three runs the development of pH and peptide concentration (Fig. 1). The results show that the peptide concentration rose and fell as the pH moved into and out of the optimum range. These results suggest that the control of pH and of the pH-dependence of the pathways were among the first problems to be solved by the early organisms. The lowering of the pH can be explained by the formation of acids from CO in our reaction system. We demonstrated this effect in an experiment with 1 mmol of FeS, 1 mmol of NiS, 4 mmol of CO, and 0.5 μmol of CH_3SH in 10 ml of water at 100°C. After 17 days we detected 67 μmol of CH_3COOH, 600 μmol of HCOOH, and 1.3 mmol of CO_2.

The reaction mechanism has both a thermodynamic and a kinetic aspect. Thermodynamically, the formation of peptides under our hot, dilute, aqueous conditions is endergonic. Therefore the mechanism must explain energy coupling with an exergonic reaction. Mechanisms based on three exergonic reactions may be considered: (i) the oxidative conversion of CO to CO_2 via COS; (ii) the hydrolytic conversion of CO to HCOOH; and (iii) the formation of acetic acid from CH_3SH and CO via activated acetic acid. These possibilities cannot be assumed to be jointly exhaustive or mutually exclusive.

The first possibility of an energy coupling with the oxidative conversion of CO to CO_2 via COS (Fig. 2A) involves a thiazolidinedione or an oxazolidinedione (Leuchs anhydride) or its 2-thio derivative as the species suffering nucleophilic attack; this reaction has the advantage of being nonanionic. This mechanism is supported by the formation of COS in this reaction system (*1*), and by the observation that dipeptides were formed (data not shown), if under otherwise identical conditions we replaced CO and H_2S (or CH_3SH) by COS, but not in the absence of NiS and FeS.

The second possibility of an energy coupling with the conversion of CO to HCOOH is supported by the facile formation of formic acid in our experiments at rates that seem to correspond to the rates of peptide formation. The detection of small amounts of *N*-formyl-amino acids indicates that a reaction channel from CO to HCOOH proceeds through an activated formic acid. However, a mixed anhydride (H_2N-CHR-CO-O-CO-H) as the species suffering nucleophilic attack by an amino acid may be ruled out, because it would react intramolecularly rather than intermolecularly. This inference leads us to a hypothetical mechanism in which oxazolinone acts as the nonionic species for the nucleophilic attack by the amino acid, as shown in Fig. 2B.

The third possibility of an energy coupling with the formation of acetic acid is supported by the detection of acetic acid under our reaction conditions in the presence of CH_3SH, by the previously demonstrated (*2*) formation of thioacetic acid (CH_3-COSH) or its methyl ester (CH_3-CO-SCH_3), and by the formation of small amounts of the *N*-acetyl-amino acids in our system. A mechanism to the one shown in Fig. 2B may proceed through a 2-methyl-oxazolinone. Such a mechanism could make only a small contribution, because the formation of dipeptides proceeds similarly well in the presence and absence of CH_3SH and because the rate of peptide formation is higher than the rate of acetic acid formation. We did not detect any dipeptides, if under otherwise identical conditions CO was replaced by 500 μmol of CH_3COSH or CH_3COSCH_3. This result means that neither of these two activated compounds is situated in a reaction channel to the peptides.

The problem of the endergonic nature of peptide formation in dilute aqueous solutions, calculated for glycine to have a maximum around 100°C (*5*), has previously been approached by changing the thermodynamic conditions with heating in dry condition (*6*), drying/wetting cycles (*7, 8*), or high salt concentrations (*9*). Under the dilute aqueous conditions most relevant for the origin of life, activation of the amino acids by coupling with hydrolysis reactions (*10*) notably of inorganic polyphosphates (*11*) has been suggested. It is, however, not clear how under hot aqueous conditions such hydrolytically sensitive coupling compounds, if geochemically available at all, could resist rapid equilibration. In our system the chemical potential is maintained for a long time, and under natural conditions it may be readily replenished by volcanic exhalations containing CO.

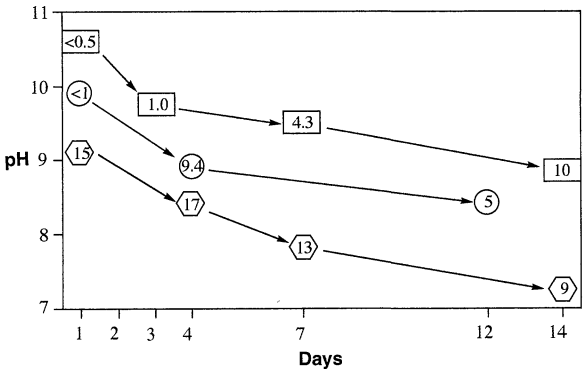

Fig. 1. Plot of development of pH and dipeptide yields over time. The numerals in circles denote the yields of LF-LF from LF; the numerals in rectangles, the yields of LY-LY from LY; and the numerals in hexagons, the yields of G-G. The standard deviation of the yields of G-G is up to ±11% (four repeats).

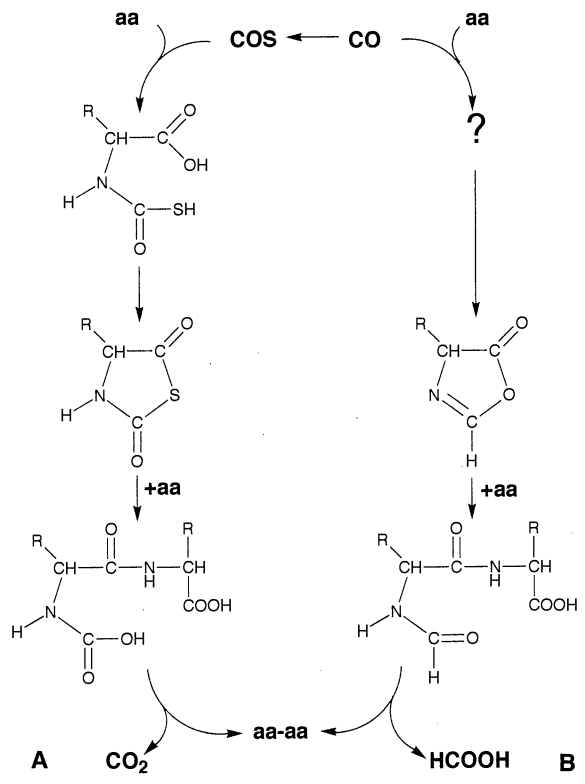

Fig. 2. Notional representation of alternative ligand sphere reaction mechanisms. The symbol "aa" represents an amino acid of the formula $RCHNH_2$-COOH, "aa-aa" represents its dipeptide and "?" an unknown intermediate, perhaps a 2-mercapto-exazolinone. In the cyclic intermediate of mechanism A positions 1 and 2 may be occupied alternatively by O and CO or by O and CS, respectively.

Kinetically the (Fe,Ni)S catalyst in our system promotes the productive reaction channel to peptides as compared to other nonproductive channels. It means that the reaction occurs in the ligand sphere of the sulfide mineral. Previously, copper ions have been used as catalyst for peptide formation in the presence of high salt concentrations (*9*). However, under anaerobic conditions with even a small sulfide activity, copper ions cannot exist.

Most prior attempts to produce peptides under primordial conditions have been beset by the formation of large amounts of unreactive diketopiperazines (*7, 12*). For example, in drying-wetting cycle experiments with glycine on montmorillonite, the molar ratio of diketopiperazine to diglycine was more than 4:1 (*13*). The formation of diketopiperazines was so far only suppressed, if the amino acid was activated in the form of a Leuchs anhydride, which required organic activation agents such as carbodiimides (*14*). In our system the diketopiperazines form minor byproducts. For example, in run 25, the amount of the diketopiperazine was 3.5 ± 0.5 μmol; while in run 13 the amount of the diketopiperazine of tyrosine is 5 μmol after 1 day and 8 μmol after 4 days (*15*). Both mechanisms shown in Fig. 2 would disfavor the formation of the diketopiperazine. They would also explain the racemization by resonance-stabilized enolization.

Our result supports the theory of a thermophilic origin of life with a primordial surface metabolism on transition metal sulfide minerals. It means that a continuously recycling library of peptides was generated on the surfaces of a library of (Fe,Ni)S structures. It raises the possibility that CO and Ni had a much greater role in the primordial metabolism than in any of the known extant metabolisms. All known extant organisms are found in habitats with low activities of CO and Ni. This could explain why they resorted to the formation of CO from CO_2 and to the elimination of nickel from many enzymes (*16*).

References and Notes

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2. In a typical run a 120-ml serum bottle was charged with 278 mg (1 mmol) of $FeSO_4 \cdot 7H_2O$, 262 mg (1 mmol) of $NiSO_4 \cdot 6H_2O$ and 82.5 mg (0.5 mmol) of phenylalanine, closed with a silicon stopper (Bender und Hobein, Kleinostheim), deaerated and subsequently charged with 8 ml of deaerated and Ar-saturated water and with a solution of 480 mg (2 mmol) of $Na_2S \cdot 9H_2O$ in 2 ml of water for the precipitation of the sulfides, 1.05 bar CO gas (CO 2.0 Messer Griesheim), 12 ml (0.5 mmol) of CH_3SH gas, and 0.4 ml of 4N NaOH for adjusting the pH. In the absence of CH_3SH 2.5 mmol of Na_2S were used. The reaction was carried out at 100°C for 1 day, after which the pH was 9. All solutions were prepared from doubly distilled water, which was boiled and cooled under a stream of nitrogen. The peptides were identified and quantified by HPLC, using Merck-Hitachi Pump L-7100, with the columns Nucleosil 10C18 for phenylalanine and tyrosine and Nucleosil 10SA for glycine, and with the ultraviolet detector Merck-Hitachi L-7400 set to 258 nm for phenylalanine, 274 nm for tyrosine and 195 or 215 nm for glycine of runs 25 or 26 to 34, respectively). For phenylalanine and tyrosine elution was carried out for 0 to 2 min with H_2O/1 per mil H_3PO_4, a linear gradient from 2 to 42 min, and methanol/1 per mil H_3PO_4 for 42 to 45 min. For glycine isocratic elution was carried out with H_2O/1 per mil H_3PO_4. The dipeptides of phenylalanine and tyrosine (and the tripeptide of tyrosine) were additionally identified by the detection of the molecular ion by HPLC-MS-ESI, using Hewlett-Packard Series 1100 (HPLC) and LCQ Finnigan Mat (MS), an RP18 5-μm column and a linear gradient of 0 to 70% CH_3CN.
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17. The work was supported by the Deutsche forschungsgemeinschaft. We thank H. Simon and A. Bacher for providing the laboratory facilities for carrying out this work and for their continued support, M. Urzinger for HPLC-MS, C. Riemer for synthesis of dipeptides, U. Zachariae and R. Heidenreich for laboratory assistance, and O. Kandler and H. Kessler for valuable advice.

15 April 1998; accepted 9 June 1998

Circular Polarization in Star-Formation Regions: Implications for Biomolecular Homochirality

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Strong infrared circular polarization resulting from dust scattering in reflection nebulae in the Orion OMC-1 star-formation region has been observed. Circular polarization at shorter wavelengths might have been important in inducing chiral asymmetry in interstellar organic molecules that could be subsequently delivered to the early Earth by comets, interplanetary dust particles, or meteors. This could account for the excess of L–amino acids found in the Murchison meteorite and could explain the origin of the homochirality of biological molecules.

The origin of the homochirality of biological molecules (living systems use almost exclusively L–amino acids and D-sugars) has been a puzzle since the effect was discovered in the 19th century. Homochirality may be a prerequisite for the origin of life (*1*). A number of processes have been proposed that might operate soon after the formation of Earth to produce an enantiomeric excess in prebiotic organic molecules (*2*, *3*), including the action of circular polarization (CP) from the daylight sky and effects caused by the parity-violating aspect of the electroweak interaction. These are small effects and would require amplification by factors $\leq 10^{17}$ (*2*, *4*) to account for homochirality. The difficulty with any proposed Earth-based mechanism led Bonner (*3*), in a detailed review of the origin of homochirality, to suggest an extraterrestrial origin. Support for this view comes from the discovery of an excess of L–amino acids in the Murchison meteorite (*5*, *6*).

In the laboratory, high levels of enantiomeric excess in racemic substances can be produced by asymmetric photolysis by circularly polarized light (*3*). Twenty percent enantiomeric excess has been demonstrated in the laboratory (*7*) for 99% photolysis of camphor. The excess can be increased, by increasing the fraction of material photolyzed, but it will be reduced in proportion to the CP for CP of less than 100%. Astronomical sources of CP might

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